

**AMENDMENTS TO THE CLAIMS**

Please amend claims 1, 3-4, 6-9, 11-13, 29-30, 54, 56-67, 60-62, and 64, cancel claims 5, 10, 31, and 63, and add new claims 65-81, as shown in the following listing of claims, which will replace all prior versions and listings of claims in the application. Please cancel claims 5, 10, 31, and 63 without prejudice to their pursuit in an appropriate divisional or continuation application. Claims 14-28 and 32-41 are withdrawn. Claims 1-4, 6-9, 11-13, 29-30, 54-62, and 64-81 are currently in the application.

**Listing of claims:**

1 (currently amended). A method for the production of cell-cycle-specific differentiated hematopoietic cells comprising:

a) culturing purified bone marrow stem cells for cycle initiation cells, from resting ~~[[state]]~~ state, under conditions that promote synchronous progression through the cell cycle;

~~b) selecting at least one desired cell cycle specific hematopoietic cell type to be differentiated from the synchronous purified bone marrow stem cells upon contact with at least one growth factor or cytokine at a phase of the cell cycle favoring a specific differentiation pathway for the at least one desired cell cycle specific hematopoietic cell type;~~

~~c) determining the phase of the cell cycle favoring the specific differentiation pathway for the at least one desired cell cycle specific hematopoietic cell type, and selecting the at least one growth factor or cytokine to promote the specific differentiation pathway or to produce a plurality of cells of the at least one desired cell cycle specific hematopoietic cell type to be differentiated from the synchronous purified bone marrow stem cells upon with at least one growth factor or cytokine at a phase of the cell cycle with the at least one growth factor or cytokine at the pre-determined phase of the cell cycle;~~

[[d]]b)contacting the ~~synchronous-purified bone marrow stem cells~~ of step a) with ~~[[the]]~~ at least one growth factor or cytokine ~~at the predetermined phase of the cell cycle~~ in mid-S phase; and

[[e]]c) subculturing the cells of step b) until ~~cell cycle specific differentiated hematopoietic cells are produced, wherein a plurality of the cell cycle specific differentiated hematopoietic cells have the at least one desired cell cycle specific hematopoietic cell type produced.~~

2 (previously presented). The method of claim 1, wherein the at least one growth factor or cytokine comprises G-CSF, GM-CSF, or steel factor.

3 (currently amended). The method of claim 1, wherein ~~culturing the cells under conditions that promote synchronous progression through the cell cycle~~ step a) comprises culturing the cells in the presence of steel factor, thrombopoietin, and FLT3-ligand.

4 (currently amended). The method of claim 1, wherein ~~the step of subculturing the cells is carried about~~ step c) is carried out for about 14 days.

5 (canceled).

6 (currently amended). The method of claim ~~[[5,]]~~ 1, wherein mid-S phase occurs about 32 hours after initiation of the culturing of the stem cells under conditions that promote synchronous progression through the cell cycle.

7 (currently amended). The method of claim 1, wherein a plurality of the differentiated hematopoietic cells ~~comprise~~comprises megakaryocytes.

8 (currently amended). The method of claim 1, wherein a plurality of the differentiated hematopoietic cells ~~comprise~~comprises platelets.

9 (currently amended). The method of claim 1, wherein a plurality of the differentiated hematopoietic cells ~~comprise~~comprises proliferative granulocytes.

10 (canceled).

11 (currently amended). The method of claim ~~[[10,]]~~ 68, wherein late S phase occurs about 40 hours after initiation of the culturing of the stem cells under conditions that promote synchronous progression through the cell cycle.

12 (currently amended). The method of claim ~~[[1,]]~~ 68, wherein the differentiated hematopoietic cells comprise ~~mature (non-proliferative)~~ mature or non-proliferative granulocytes.

13 (currently amended). The method of claim 1, further ~~comprising~~ comprising:  
d) \_\_\_\_\_ isolating the differentiated hematopoietic cells from the subculture.

14 (withdrawn). A method of treating a subject having cytopenia comprising administering to the subject a therapeutically effective amount of the differentiated hematopoietic cells produced according to the methods of claim 1.

15 (withdrawn). A method of preventing cytopenia in a subject comprising administering to the subject a therapeutically effective amount of the differentiated hematopoietic cells produced according to the methods of claim 1.

16 (withdrawn). The method of any one of claims 14-15, wherein the subject has or is at risk for developing cytopenia associated with cancer chemotherapy or radiation therapy.

17 (withdrawn). The method of any one of claims 14-15, wherein the subject has or is at risk for developing cytopenia associated with a bone marrow transplant.

18 (withdrawn). The method of any one of claims 14-15, wherein the cytopenia is thrombocytopenia.

19 (withdrawn). The method of any one of claims 14-15, wherein the cytopenia is granulocytopenia.

20 (withdrawn). Hematopoietic cells produced by the methods of any one of claims 1, 14, or 15.

- 21 (withdrawn). The hematopoietic cells of claim 20, which are macrophages.
- 22 (withdrawn). The hematopoietic cells of claim 20, which are platelets.
- 23 (withdrawn). The hematopoietic cells of claim 20, which are proliferative granulocytes.
- 24 (withdrawn). The hematopoietic cells of claim 20, which are mature (non-proliferative) granulocytes.
- 25 (withdrawn). A method for the production of differentiated hematopoietic cells comprising:
- a) culturing bone marrow stem cells under conditions that promote synchronous progression through the cell cycle;
  - b) contacting the cells with at least one growth factor or cytokine at a predetermined phase of the cell cycle, wherein:
    - i) the growth factor comprises G-CSF, GM-CSF, or steel factor; and
    - ii) the predetermined phase of the cell cycle is mid-S phase or late S phase;
  - c) subculturing the cells until differentiated hematopoietic cells are produced; and
  - d) isolating the differentiated hematopoietic cells from the subculture.

26 (withdrawn). A method of treating a subject having cytopenia comprising administering to the subject a therapeutically effective amount of the isolated differentiated hematopoietic cells produced according to the method of claim 25.

27 (withdrawn). A method preventing cytopenia in a subject comprising administering to the subject a therapeutically effective amount of the differentiated hematopoietic cells produced according to the method of claim 25.

28 (withdrawn). Isolated hematopoietic cells produced by the method of claim 25.

29 (currently amended). The method of claim 1, wherein the predetermined phase of the cell cycle comprises a differentiation hotspot favoring a specific differentiation pathway at the predetermined phase of the cell cycle.

30 (currently amended). The method of claim 1, wherein the predetermined phase of the cell cycle comprises a reversible differentiation hotspot favoring a specific differentiation pathway at the predetermined phase of the cell cycle, wherein a differentiated cell arises from a stem cell.

31. (canceled)

32 (withdrawn). A method for the production of cell cycle specific differentiated hematopoietic cells comprising:

- a) culturing bone marrow stem cells under conditions that promote synchronous progression through the cell cycle by:
  - i) providing purified bone marrow stem cells; and
  - ii) culturing the purified bone marrow stem cells for cycle initiation from resting state in medium comprising steel factor (FTS), FLT-3 ligand, and thrombopoietin to obtain a culture predominantly comprising synchronous bone marrow stem cells;
- b) contacting the synchronous bone marrow stem cells with an inductive differentiation medium at a predetermined phase of the cell cycle, wherein:
  - i) the predetermined phase of the cell cycle is mid-S phase or late S phase; and
  - ii) the inductive differentiation medium comprises G-CSF, GM-CSF, and steel factor (FTS); and
- c) subculturing the cells until cell cycle specific differentiated hematopoietic cells are produced.

33 (withdrawn). The method of claim 32, wherein the predetermined phase of the cell cycle is mid-S phase and wherein mid-S phase occurs about 32 hours after initiation of the culturing of the stem cells under conditions that promote synchronous progression through the cell cycle.

34 (withdrawn). The method of claim 32, wherein the predetermined phase of the cell cycle is late S phase and wherein late S phase occurs about 40 hours after initiation of

the culturing of the stem cells under conditions that promote synchronous progression through the cell cycle.

35 (withdrawn). The method of claim 32, wherein the step of subculturing the cells is carried about for about 14 days.

36 (withdrawn). The method of claim 32, further comprising isolating the differentiated hematopoietic cells from the subculture.

37 (withdrawn). The method of claim 32, wherein the differentiated hematopoietic cells comprise megakaryocytes.

38 (withdrawn). The method of claim 32, wherein the differentiated hematopoietic cells comprise platelets.

39 (withdrawn). The method of claim 32, wherein the differentiated hematopoietic cells comprise proliferative granulocytes.

40 (withdrawn). The method of claim 32, wherein the differentiated hematopoietic cells comprise mature (non-proliferative) granulocytes.

41 (withdrawn). The method of claim 32, wherein the bone marrow stem cells comprise Lineage<sup>negative</sup>Rhodamine<sup>low</sup>Hoescht<sup>low</sup> (LRH) cells.



42. – 53. (canceled)

54 (currently amended). A method for the production of ~~cell cycle specific cell~~  
cycle-specifically differentiated hematopoietic cells comprising:

a) culturing purified bone marrow stem ~~cells for cycle initiation cells~~,  
from resting ~~[[state]] state~~, under conditions that promote synchronous progression through  
the cell cycle;

b) contacting the cells of step a) with at least one growth factor or  
cytokine at a predetermined phase of the cell cycle; and

c) subculturing the cells of step b) until ~~cell cycle specific~~ differentiated  
hematopoietic cells are produced,

wherein:

i) the predetermined phase of the cell cycle is mid-S phase and the differentiated  
hematopoietic cells comprise megakaryocytes, platelets, or proliferative granulocytes; or

ii) the predetermined phase of the cell cycle is late S phase and the differentiated  
hematopoietic cells comprise ~~mature (non-proliferative)~~ mature or non-proliferative  
granulocytes.

55 (previously presented). The method of claim 54, wherein the at least one growth  
factor or cytokine comprises G-CSF, GM-CSF, or steel factor.

56 (currently amended). The method of claim 54, wherein ~~culturing the cells under conditions that promote synchronous progression through the cell cycle step a)~~ comprises culturing the cells in the presence of steel factor, thrombopoietin, and FLT3-ligand.

57 (currently amended). The method of claim 54, wherein ~~the step of subculturing the cells is carried about~~ step c) is carried out for about 14 days.

58 (previously presented). The method of claim 54, wherein the predetermined phase of the cell cycle is mid-S phase and mid-S phase occurs about 32 hours after initiation of the culturing of the stem cells under conditions that promote synchronous progression through the cell cycle.

59 (previously presented). The method of claim 54, wherein the predetermined phase of the cell cycle is late S phase and late S phase occurs about 40 hours after initiation of the culturing of the stem cells under conditions that promote synchronous progression through the cell cycle.

60. (currently amended). The method of claim 54, further ~~comprising~~ comprising:

d) isolating the differentiated hematopoietic cells from the subculture.

61 (currently amended). The method of claim 54, wherein the predetermined phase of the cell cycle comprises a differentiation hotspot favoring a specific differentiation pathway at the predetermined phase of the cell cycle.

62 (currently amended). The method of claim 54, wherein the predetermined phase of the cell cycle comprises a reversible differentiation hotspot favoring a specific differentiation pathway at the predetermined phase of the cell cycle, wherein a differentiated cell arises from a stem cell.

63. (canceled)

64 (previously presented). The method of claim 54, wherein the bone marrow stem cells comprise Lineage<sup>negative</sup>Rhodamine<sup>low</sup>Hoescht<sup>low</sup> (LRH) cells.

65 (new). The method of claim 1, wherein the bone marrow stem cells comprise Lineage<sup>negative</sup>Rhodamine<sup>low</sup>Hoescht<sup>low</sup> (LRH) cells.

66 (new). The method of claim 1, wherein prior to step a), the bone marrow stem cells are isolated by fluorescence activated cell sorting (FACS).

67 (new). The method of claim 54, wherein prior to step a), the bone marrow stem cells are isolated by fluorescence activated cell sorting (FACS).

68 (new). A method for the production of differentiated hematopoietic cells comprising:

a) culturing purified bone marrow stem cells, from resting state, under conditions that promote synchronous progression through the cell cycle;

b) contacting the synchronous cells of step a) with at least one growth factor or cytokine in late S phase; and

c) subculturing the cells of step b) until differentiated hematopoietic cells are produced.

69 (new). The method of claim 68, further comprising:

d) isolating the differentiated hematopoietic cells from the subculture.

70 (new). The method of claim 68, wherein the late S phase of the cell cycle comprises a differentiation hotspot favoring a specific differentiation pathway at the predetermined phase of the cell cycle.

71 (new). The method of claim 68, wherein the predetermined phase of the cell cycle comprises a reversible differentiation hotspot favoring a specific differentiation pathway at the predetermined phase of the cell cycle, wherein a differentiated cell arises from a stem cell.

72 (new). The method of claim 68, wherein the bone marrow stem cells comprise Lineage<sup>negative</sup>Rhodamine<sup>low</sup>Hoescht<sup>low</sup> (LRH) cells.

73 (new). The method of claim 68, wherein prior to step a), the bone marrow stem cells are isolated by fluorescence activated cell sorting (FACS).

74 (new). A method for the production of cell cycle-specifically differentiated hematopoietic cells comprising:

a) culturing purified bone marrow stem cells, from resting state, under conditions that promote synchronous progression through the cell cycle, to yield a plurality of synchronously progressing bone marrow stem cells;

b) contacting the synchronously progressing bone marrow stem cells of step a) with at least one growth factor or cytokine at a specific phase of the cell cycle, wherein contacting the purified bone marrow stem cells with the growth factor or cytokine at the specific phase of the cell cycle promotes a specific differentiation pathway to yield a plurality of cells having a cell cycle-specifically differentiated hematopoietic cell type; and

c) subculturing the cells of step b) until cell cycle-specifically differentiated hematopoietic cells are produced, wherein a plurality of the cell cycle-specifically differentiated hematopoietic cells has the at least one desired cell cycle-specifically differentiated hematopoietic cell type.

75 (new). The method of claim 74, further comprising:

d) isolating the differentiated hematopoietic cells from the subculture.

76 (new). The method of claim 74, wherein the specific phase of the cell cycle in step b) comprises a differentiation hotspot favoring a specific differentiation pathway at the predetermined phase of the cell cycle.

77 (new). The method of claim 74, wherein the predetermined phase of the cell cycle comprises a reversible differentiation hotspot favoring a specific differentiation pathway at the predetermined phase of the cell cycle, wherein a differentiated cell arises from a stem cell.

78 (new). A method for the production of cell cycle-specifically differentiated hematopoietic cells comprising:

- a) providing a culture of synchronously cycling bone marrow stem cells;
- b) contacting the synchronously cycling bone marrow stem cells of step a) with at least one growth factor or cytokine at a specific phase of the cell cycle, wherein the contacting of the purified bone marrow stem cells with the growth factor or cytokine at the specific phase of the cell cycle promotes a specific differentiation pathway to yield a plurality of cells having a cell cycle-specifically differentiated hematopoietic cell type; and
- c) subculturing the cells of step b) to produce differentiated hematopoietic cells, wherein a plurality of the differentiated hematopoietic cells has the at least one desired cell cycle-specifically differentiated hematopoietic cell type.

79 (new). The method of claim 78, further comprising:

- d) isolating the differentiated hematopoietic cells from the subculture.

80 (new). The method of claim 78, wherein the specific phase of the cell cycle in step b) comprises a differentiation hotspot favoring a specific differentiation pathway at the predetermined phase of the cell cycle.

81 (new). The method of claim 78, wherein the predetermined phase of the cell cycle comprises a reversible differentiation hotspot favoring a specific differentiation pathway at the predetermined phase of the cell cycle, wherein a differentiated cell arises from a stem cell.